Journal of Surgical Oncology 61:106–110 (1996)

Significance of Proliferating Cell Nuclear Antigen (PCNA) Expression in Gastric Cancer in Relation to Lymph Node Metastasis

HIROSHI ISOZAKI, MD, KUNIO OKAJIMA, MD, TADASHI ICHINONA, MD, KEIZO FUJII, MD, EIJI NOMURA, MD, NOBUYUKI IZUMI, MD, AND YOSHI TAKEDA, MD
From the Department of General and Gastroenterological Surgery, Osaka Medical College, Osaka, Japan

Proliferating cell nuclear antigen (PCNA) in gastric cancer was evaluated in relation to lymph node metastasis. A total of 125 gastric cancer patients who underwent gastrectomy were studied immunohistochemically. The PCNA-positive rate of the primary lesion with lymph node metastasis (47.6%) was significantly higher than that in those without metastasis (24.3%, $P < 0.0001$). The PCNA-positive rate of early gastric cancer was significantly higher in lesions with lymph node metastasis (36.9%) than in lesions without lymph node metastasis (14.7%). However, there was no significant difference between lesions with and without lymph node metastasis in advanced gastric cancer. In addition, the PCNA-positive rate in metastatic lesions (44.6%) was significantly higher than that in the primary lesion (40.0%, $P = 0.001$). It is concluded that gastric cancer with higher tumor growth activity has a higher rate of lymph node metastasis. Cancer cells in the metastatic foci of lymph node have a higher proliferating activity than that in the primary lesion. © 1996 Wiley-Liss, Inc.

KEY WORDS: **gastric cancer, early gastric cancer, lymph node metastasis, proliferating cell nuclear antigen (PCNA)**

INTRODUCTION

It is well known that gastric cancer patients with lymph node metastasis have poorer prognosis than those without lymph node metastasis. In general, gastric cancer with deeper depth of invasion has a higher rate of lymph node metastasis. However, the extent of lymph node metastasis can differ even in cancers with the same depth of invasion, and this is thought to be due to differences in the biological behavior of cancer. The proliferating cell nuclear antigen (PCNA) [1] is an auxiliary protein of DNA polymerase δ, which is synthesized between the late G_1 and S phases. PCNA has also been recognized as a useful index of cell proliferating activity [2]. In the present study, the significance of PCNA in gastric cancer was evaluated, focusing on the relation with lymph node metastasis.

MATERIALS AND METHODS

Patients

A total of 125 cases of gastric cancer were randomly selected among the cases who underwent radical gastrectomy in our department between 1978 and 1993 and in whom the evaluation of PCNA staining was possible. These patients consisted of 86 males (average age, 59 years) and 39 females (average age, 55.3 years). In this group, the 63 early gastric cancer patients, in which tumor involved the mucosa or submucosa of the gastric wall, comprised 16 patients with, and 47 patients without, lymph node metastasis, and the 62 advanced gastric cancer patients, in which tumor invaded the muscularis propria or deeper, comprised 41 patients with, and 21 patients without, lymph node metastasis. The PCNA expression of the primary gastric lesion in those patients was evaluated according to the clinicopathological findings. A comparison of the PCNA expression in the primary and metastatic lymph node lesions was performed in the 36 patients with lymph node metastasis.

Accepted for publication August 31, 1995.

Address reprint requests to Hiroshi Isozaki, M.D., Department of General and Gastroenterological Surgery, Osaka Medical College, 2-7, Daigaku-machi, Takatsuki, Osaka 569, Japan.

© 1996 Wiley-Liss, Inc.

Immunohistochemical Staining by Indirect Enzyme Labeled Antibody Method and Evaluation of PCNA Expression

Tissue sections, 4 μm in thickness, were prepared from a block taken from the primary lesions and metastatic lymph nodes embedded in paraffin following fixation in 10% formalin for 2–5 days. These sections were then dewaxed in xylene and rehydrated through alcohol, after which endogenous peroxidase was inhibited, and normal sheep serum applied for 30 minutes to reduce nonspecific antibodies. Immunohistochemical staining by the indirect enzyme-labeled antibody method was performed, using the anti-PCNA monoclonal antibody (PC10, Dako A/S, Copenhagen, Denmark) [3].

Assessment of PCNA expression was carried out by one examiner who was not informed of the clinicopathological profile of the patients. Although there was some heterogeneity of PCNA expression, the number of PCNA-positive cancer cells in every 500 cancer cells at a site with a relatively high degree of staining in the proliferating part of a tumor was calculated and expressed as the PCNA positive rate:

PCNA-positive rate = PCNA-positive cancer cells/500 cancer cells × 100 (%).

Clinicopathological findings were described according to the Japanese Classification of Gastric Carcinoma [4] issued by the Japanese Research Society for Gastric Cancer (JRSGC). The histology of the cancers was divided into two types: differentiated type (papillary adenocarcinoma, well-differentiated tubular adenocarcinoma, and moderately differentiated tubular adenocarcinoma) and poorly differentiated type (poorly differentiated adenocarcinoma, signet ring cell adenocarcinoma, and mucinous adenocarcinoma).

The nuclear DNA ploidy pattern of the primary lesions was determined by flow cytometer. Paraffin-embedded tissue from the primary lesion was excised so the thickness would be 50 μm. After the block was treated according to the method of Schutte et al. [5], it was subjected to flow cytometry. In 124 patients with coefficients of variation below 8.0, those with a single peak in G_0 and G_1 were classified as diploid and those with multiple peaks in G_0 and G_1 phases as aneuploid.

The statistical significance of differences was evaluated by the unpaired Student's *t*-test in which PCNA positive rate of primary lesions were compared, and the paired Student's *t*-test in which the PCNA positive rate between primary lesion and metastatic lesion was compared. The correlation between the PCNA positive rate and the ratio (%) of S phase by flow cytometry was evaluated by Pearson's correlation coefficient.

RESULTS

The mean ± SD of the PCNA positive rate of primary lesions was 35.3 ± 20.7 and that of the ratio of S phase by flow cytometry was 26.8 ± 16.7. However, no correlation between the PCNA positive rate and the S phase ratio was recognized by Pearson's correlation coefficient ($r \pm 0.0857$, $P = 0.3667$).

PCNA-Positive Rates of the Primary Lesion According to Clinicopathological Findings

The PCNA-positive rate of the primary lesion was significantly higher in advanced gastric cancer than that in early gastric cancer. The PCNA-positive rate in lesions with a maximum dimension of 40 mm or more was significantly higher than that in lesions with a maximum dimension of <40 mm. The PCNA-positive rate in lesions with lymph node metastasis (47.6%) was significantly higher than that in those without metastasis (24.3%, $P < 0.0001$), and the PCNA-positive rate of in lesion with lymphatic vessel or vascular involvement had higher rates than those without involvement. Lesions with aneuploid DNA patterns had higher PCNA-positive rates than diploid type cases. However, there was no statistically significant difference between histological types (Table I).

PCNA-Positive Rate of Primary Lesion According to Lymph Node Metastasis

The PCNA-positive rate of early gastric cancer was significantly higher in lesions with lymph node metastasis (36.9%) than in lesions without lymph node metastasis (14.7%). Moreover, in lesions confined to the mucosa, and also in lesions invading the submucosal layer, those with metastasis had significantly higher PCNA-positive rates than those without metastasis. However, there was no significant difference between lesions with and without lymph node metastasis in advanced gastric cancer. It was also recognized that the PCNA-positive rate in lesions with lymph node metastasis was significantly higher than those without metastasis, the diploid DNA group, the aneuploid DNA group, the differentiated type group and poorly differentiated type group (Table II).

PCNA-Positive Rate of Primary and Metastatic Lesions

In the 36 patients in this series with lymph node metastasis, the PCNA-positive rate in the metastatic lesion (44.6%) was significantly higher than that in the primary lesion (40.0%, $P = 0.0012$, Table III). Even when these patients were divided into the early cancer group ($n = 20$) or the advanced cancer group ($n = 16$), the PCNA-positive rate of the metastatic lesion was significantly higher than that of the primary lesion in each group.

DISCUSSION

In gastric cancer, lymph node metastasis is closely related to the depth of cancerous invasion. Gastric cancer confined to the mucosa (m-cancer) has a low rate (0.6–11%) of lymph node metastasis, but when cancer invades

TABLE I. PCNA-Positive Rate in Primary Lesion of Gastric Cancer According to Clinicopathological Findings

Variable	PCNA-positive rate % (n)[a]	
Early gasric cancer	20.3 ± 13.5 (63)	*$P < 0.0001$
Advanced gastric cancer	49.7 ± 15.4 (62)	
Deepest layer of invasion		
Mucosa (m)	14.5 ± 10.0 (29)	*$P = 0.0009$
Submucosa (sm)	25.3 ± 14.2 (34)	*$P < 0.0001$
Muscularis proporia (mp)	50.7 ± 14.0 (15)	$P = 0.4430$
Subserosa (ss)	57.1 ± 26.4 (8)	$P = 0.2347$
Serosa (se)	47.4 ± 14.8 (38)	
Invasion to neighboring organ	62.2 (1)	
Maximum dimension of tumor		
<20 mm	24.7 ± 21.6 (6)	$P = 0.2800$
<40 mm	26.5 ± 20.1 (45)	*$P = 0.0004$
<60 mm	41.4 ± 16.2 (30)	$P = 0.8184$
>60 mm	40.4 ± 20.8 (44)	
Lymph node metastasis		
Negative	24.3 ± 18.3 (68)	*$P < 0.0001$
Positive	47.6 ± 15.4 (57)	
Lymphatic vessel involvement		
Negative	19.1 ± 14.9 (43)	*$P < 0.0001$
Positive	43.1 ± 18.2 (81)	
Vascular involvement		
Negative	30.3 ± 21.5 (88)	*$P < 0.0001$
Positive	45.7 ± 13.1 (37)	
Histological type		
Well differentiated type	32.8 ± 18.7 (60)	$P = 0.2800$
Poorly differentiated type	36.7 ± 22.2 (65)	
DNA ploidy pattern		
Diploid	28.9 ± 20.7 (76)	*$P < 0.0001$
Aneuploid	43.8 ± 17.6 (48)	

[a]PCNA-positive rate, mean ± SD; statistical analysis by unpaired Student's *t*-test. ( ), Number of primary lesions.
*Significant.

TABLE II. PCNA-Positive Rate in Primary Lesion of Gastric Cancer According to Lymph Node Metastasis

	Lymph node metastasis[a]		
Variable	Negative % (n)	Positive % (n)	*P* value
Early gastric cancer	14.7 ± 9.1 (47)	36.9 ± 10.2 (16)	*$P < 0.0001$
Advanced gastric cancer	45.7 ± 15.4 (21)	51.7 ± 15.2 (41)	$P = 0.1483$
Deepest layer of invasion			
Mucosa (m)	11.5 ± 7.5 (24)	29.1 ± 7.9 (5)	*$P = 0.0059$
Submucosa (sm)	18.0 ± 9.6 (23)	40.5 ± 9.3 (11)	*$P < 0.0001$
Musclaris propria (mp)	42.2 ± 12.9 (6)	56.2 ± 12.2 (9)	$P = 0.0625$
Subserosa (ss)	56.8 ± 24.6 (4)	57.4 ± 19.0 (4)	$P = 0.9695$
Serosa (se) or deeper	49.4 ± 12.1 (11)	49.4 ± 15.5 (28)	$P = 0.2131$
Histological type			
Well differentiated type	24.7 ± 16.5 (37)	45.8 ± 14.3 (23)	*$P < 0.0001$
Poorly differentiated type	23.7 ± 20.5 (31)	48.8 ± 16.3 (34)	*$P < 0.0001$
DNA ploidy pattern			
Diploid	19.5 ± 16.1 (48)	45.0 ± 16.0 (28)	*$P < 0.0001$
Aneuploid	34.2 ± 17.7 (19)	50.0 ± 14.7 (29)	*$P < 0.0001$

[a]PCNA-positive rate, mean ± SD; statistical analysis by unpaired Student's t-test. ( ), Number of primary lesions.
*Significant.

TABLE III. Comparison Between PCNA-Positive Rates in Primary Lesion and Metastatic Lesion of Gastric Cancer

Gastric cancer	PCNA-positive rate[a] Primary lesion %	Metastatic lesion in lymph node %	*P* value
Early gastric cancer (n = 20)	33.4 ± 7.1	39.1 ± 10.3	*$P = 0.0142$
Advanced gastric cancer (n = 16)	48.3 ± 10.9	51.4 ± 10.4	*$P = 0.0174$
Total (n = 36)	40.0 ± 11.6	44.6 ± 11.9	*$P = 0.0012$

[a]PCNA-positive rate, mean ± SD; statistical analysis by paired Student's t-test.
*Significant.

the submucosal layer (sm-cancer), the rate becomes 14.2–26.8% [6], and cancer invading beyond the proper muscle layer (mp-cancer) has a higher metastatic rate [7].

In the present study, there were significant differences in the PCNA-positive rate between m-cancer, sm-cancer, and mp-cancer, although no significant difference was proven in advanced cancers, and those results agree with the report of Maeda et al. [8]. However, it has been reported that gastric cancer with higher proliferating activity has a higher rate of lymph node metastasis [8–10], which was also demonstrated in this study. Yet, we could find no detailed report on the relationship between proliferating activity of cancer and lymph node metastasis in relation to the depth of invasion or various sorts of clinicopathological findings.

It was noteworthy that PCNA-positive rate may be a more valuable predictor of lymph node metastasis in early gastric cancer than in advanced cancer. Especially, even in m-cancer, lesions with lymph node metastasis had a significantly higher PCNA-positive rate than those without metastasis. Recently in Japan, limited surgery for early gastric cancer has been discussed. Thus the PCNA-positive rate of the preoperative biopsy specimen [8] could be a useful parameter for limited surgery. For advanced cancer that invaded beyond the proper muscle, the PCNA-positive rate was not a useful indicator of lymph node metastasis, although Kakeji et al. [11] reported that in the Borrmann 4 type [12] of gastric cancer in which the primary lesion had a PCNA-positive rate of >36.5% or more, there was a significantly higher rate of lymph node metastasis than cases with a rate <36.5%.

The relationship between the proliferating cell activity of metastatic lymph nodes and that of primary lesions is not well understood. Imazu et al. [13], in a study on 15 cases with gastric cancer, reported no significant differences of proliferating cell activity between the metastatic and primary lesion. However, in the present study, the proliferating cell activity of the metastatic lesion was significantly higher than that of the primary lesion. In other words, cancer cells with more proliferating activity might have greater potential for metastasis, even in the same cancerous lesion.

The correlation between the immunohistologic assessment by the PCNA positive rate in solid tumors and the assessment by flow cytometry was reported to be controversial because of the heterogeneity of PCNA staining. The PCNA-positive rate correlated with the S + G_2M fraction in a study on lymphoma [14], whereas no correlation was seen in similar studies of gastric cancer [15], hemangiopericytoma [16], or cancer of the ampulla of Vater [3]. In the present study, we calculated the PCNA-positive rate in the area with a relatively high degree of staining in the tumor, but no correlation between the PCNA positive rate and the S phase fraction ratio was recognized.

In conclusion, gastric cancer with higher tumor growth activity has a higher rate of lymph node metastasis. Moreover, cancer cells in the metastatic lymph nodes revealed higher proliferating activity than in the primary lesion.

ACKNOWLEDGMENTS

The authors thank Professor J. Patrick Barron of the International Medical Communications Center of Tokyo Medical College for his language review of the manuscript.

REFERENCES

1. Miyachi K, Fritzler MJ, Tan EM: Autoantibody to a nuclear antigen in proliferating cells. J Immunol 121:2228–2234, 1978.
2. Takasaki Y, Deng JS, Tan EM: A nuclear antigen associated with cell proliferation and blast transformation: Its distribution in synchronized cells. J Exp Med 154:1899–1909, 1981.
3. Isozaki H, Okajima K, Ichinona T, et al.: The significance of proliferating cell nuclear antigen (PCNA) expression in cancer of the ampulla of Vater in terms of prognosis. Surgery Today 24:494–499, 1994.
4. Japanese Research Society for Gastric Cancer: Part I. Clinical, surgical, and conclusive findings. In Nishi M, Omori Y, Miwa K (eds): "Japanese Classification of Gastric Carcinoma," 1st Eng. ed. Tokyo: Kanehara, 1995, p 1–35.
5. Schutte B, Reynders MMJ, Bosman FT, et al.: Flow cytometric determination of DNA ploidy level in nuclei isolated from paraffin-embedded tissue. Cytometry 6:26–30, 1985.
6. Hioki K, Nakane Y, Yamamoto M: Surgical strategy for early gastric cancer. Br J Surg 77:1330–1334, 1990.
7. Isozaki H, Okajima K, Yamada S, et al.: Proximal subtotal gastrectomy for the treatment of carcinoma of the upper third of the stomach: Its indications based on lymph node metastasis and perigastric lymphatic flow. Surgery Today 25:21–26, 1995.
8. Maeda K, Chung Y, Onoda N, et al.: Proliferating cell nuclear antigen labeling index of preoperative biopsy specimens in gastric

carcinoma with special reference to prognosis. Cancer 73:528–533, 1993.
9. Inada T, Imura J, Ichikawa A, et al.: Proliferating activity of gastric cancer assessed by immunostaining for proliferating cell nuclear antigen. J Surg Oncol 54:146–152, 1993.
10. Mori M, Kakeji Y, Adachi Y, et al.: The prognostic significance of proliferating cell nuclear antigen in clinical gastric cancer. Surgery 113:683–690, 1993.
11. Kakeji Y, Maehara Y, Adachi Y, et al.: Proliferating activity as a prognostic factor in Borrmann type 4 gastric carcinoma. Br J Cancer 69:749–753, 1994.
12. Borrmann R: Geschwulste des Magens und des Duodenums. In Henke F, Lubarsch O (eds): "Handbuch Spez Pathol Anat und Histol IV/I." Berlin:Springer, 1926, p 812–1054.
13. Imazu H, Funabiki T, Ochiai M, et al.: A study of DNA ploidy pattern, proliferating index and proliferating cell nuclear antigen (PCNA) in primary and metastatic tumors in advanced gastric carcinoma (in Japanese). J Jpn Soc Clin Surg 54;2962–2969, 1993.
14. Wood AL, Hall PA, Shepherd NA, et al.: The assessment of proliferating cell nuclear antigen (PCNA) immunostaining in primary gastrointestinal lymphomas and its relation to histological grade, S + G_2 + M phase fraction (flow cytometric analysis) and prognosis. Histopathology 19:21–27, 1991.
15. Jain S, Filipe MI, Hall PA, et al.: Prognostic value of proliferating cell nuclear antigen in gastric carcinoma. J Clin Pathol 44:655–659, 1991.
16. Yu CC-W, Hall PA, Fletcher CDM, et al.: Haemangiopericytoma: The prognostic value of immunohistochemical staining with a monoclonal antibody to proliferating cell nuclear antigen (PCNA). Histopathology 19:29–33, 1991.